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BETA-AMYLOID PEPTIDE INHIBITORS

The present invention relates to compounds which inhibit the binding of metal ions to the N-terminal region
5 of the β-amyloid peptide. In addition, the present invention relates to pharmaceutical compositions including these compounds as the active agent, and to methods of treatment involving the administration of these compounds. The compounds of the invention are useful in the treatment
10 of Alzheimer's disease.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is characterised by the presence of distinctive lesions in the victim's brain.
15 These brain lesions include abnormal intracellular filaments called neurofibrillary tangles, and extracellular deposits of amyloid in senile, or amyloid, plaques. Amyloid deposits are also present in the walls of cerebral blood vessels of Alzheimer's patients.
20 The major constituent of amyloid plaques has been identified as a 4 kilodalton peptide (39-43 residues) called β-amyloid peptide (Aβ) (Glenner and Wong, 1984). Diffuse deposits of Aβ peptides are frequently observed in normal adult brains, whereas Alzheimer's disease brain
25 tissue is characterised by more compacted, dense-core β-amyloid plaques. These observations suggest that Aβ deposition precedes, and contributes to, the destruction of neurons that occurs in Alzheimer's disease. In further support of a direct pathogenic role for AD, β-amyloid has
30 been shown to be toxic to mature neurons both in culture and *in vivo* (Yanker et al., 1989).

Natural Aβ is derived from proteolysis from a much longer protein known as the amyloid precursor protein (APP) (Kang, J et al, 1987). The APP gene maps to chromosome 21,
35 thereby providing an explanation for the β-amyloid deposition seen at an early age in individuals with Down's syndrome, which is caused by trisomy of chromosome 21.

Aβ peptides are cleaved from APP, and then undergo aggregation to produce the insoluble toxic β-sheet structures which are found in extracellular deposits in Alzheimer's disease and Down's syndrome. Recent data

5   suggest that the aggregated peptide has redox properties and can generate reactive oxygen species, which attack enzymes and possibly cell membranes, causing neurotoxicity (Markesbery, W.R. 1997). Antitioxidants are known to protect against Aβ-induced toxicity.

10   Aβ has been shown to bind copper and iron in stoichiometric amounts, with the associated formation of reactive oxygen species such as peroxides and hydroxide radicals, which are possible sources of the neurotoxicity (Bush et al., 1998). While the formation of peroxide in

15   post-mortem samples of Alzheimer's disease brain has been observed, there was little peroxide formation in control tissue (Cherny et al., 1998). The peroxidase activity observed in the samples of Alzheimer's disease brain was abolished when treated with certain chelators (Cherny et

20   al., 1998). The formation of reactive oxygen species was accompanied by a reduction in the valence state of the metal, ie Cu(II) to Cu(I) and Fe(III) to Fe(II) (Atwood et al., 1998a). Reactive oxygen species can also lead to free radical formation on the Aβ peptide, which leads over time

25   to covalent cross-linking of the Aβ peptides (Bush et al., 1998). In addition, a number of metal ions, including Zn, Ni and Cu, have been shown to induce aggregation of Aβ (Atwood et al., 1998b). When brain tissue from both control and Alzheimer's disease-affected subjects was treated with

30   chelators which are specific for zinc and copper, there was greatly enhanced solubilisation of Aβ, with an increase of up to 700%, suggesting that zinc and copper play a role in the assembly of the Aβ deposits (Cherny et al., 1998).

Histidine residues have been implicated in the

35   binding of metal ions to Aβ peptides. For instance rat Aβ1-40, in which His13 is mutated to Arg, does not aggregate, nor does Aβ1-40 treated with diethyl

pyrocarbonate, which binds to the imidazole nitrogen of
histidine (Atwood et al., 1998). Subsequently to the
priority date of this application, it was reported that
three histidine residues in the N-terminal hydrophilic

5   region of human $A\beta$ provide primary metal binding sites, and
that the solublity of the complex between matel and $A\beta$
depends on the mode of metal binding. The authors proposed
that Cu^{2+} would protect $A\beta$ against Zn-induced aggregation
by competing with zinc ions for binding sites on the

10   histidine residues (Miura et al., 2000).

In contrast, we propose that inhibition of binding of
zinc, copper and/or iron to the $A\beta$ peptide will have
significant therapeutic value in the treatment of
Alzheimer's disease.

15   It has been reported that certain tetrapyrroles,
especially certain porphyrin and phthalocyanine compounds
inhibit conversion of normal, protease-sensitive prion
protein (PrPsen) to the protease-resistant form (PrPres)
which is implicated in the pathogenesis of transmissible

20   spongiform encephalopathies (TSEs) such as Creutzfeldt-
Jacob disease (Caughey et al., 1998), and that three of
these compounds inhibited TSE disease *in vivo* (Priola et
al., 2000). However, both metal-free and metal-complexed
tetrapyrroles were active, and the authors considered that

25   the mechanism of action involved direct interaction between
the compound and the infectious agent. Although the
authors speculated that the compounds might also be useful
in the treatment of non-prion mediated amyloid-related
conditions, such as Alzehimer's disease or Type II

30   diabetes, this was no more than speculation (Priola et al.,
2000). Moreover, all of the compounds disclosed have
multiple substitutions or the tetrapyrrole ring, whereas
the tetrapyrrole compounds of the present invention are
preferably substituted only on one of the rings.

35   It will be clearly understood that, although a number
of prior art publications are referred to herein, this
reference does not constitute an admission that any of

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these documents forms part of the common general knowledge in the art, in Australia or in any other country.

SUMMARY OF THE INVENTION

5   The present inventors have now found that zinc and copper bind predominantly to a region in the N-terminal loop of Aβ which includes a cluster of histidine residues. This finding provides the basis for the rational design or selection of inhibitors of the binding of zinc, copper
10   and/or iron to Aβ.

         Accordingly, in a first aspect the present invention provides a compound which interacts with the β-amyloid peptide in such a way that the N-terminal loop of the peptide (amino acid residues 1-15) is blocked or
15   destabilised, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

         Preferably the compound inhibits binding of Cu^{2+}, Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.
20   Preferably the compound has a conformation and polarity such that it binds to at least one, more preferably at least two, and more preferably three histidine residues in the N-terminal loop, selected from the group consisting of His6, His13 and His14. More
25   preferably the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

         The compound may have acidic groups which interact with one or more of the His residues in the N-terminal
30   loop. For example, the compound may be represented as follows:
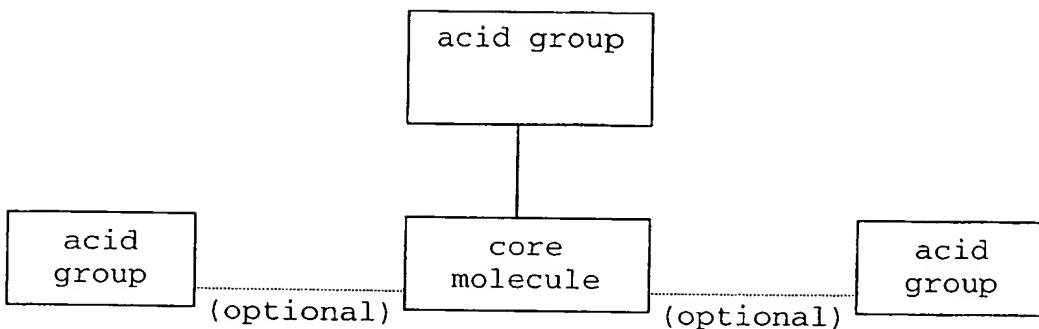
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                          ┌─────────────────┐
                          │    acid group   │
                          │                 │
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  ┌───────────┐          ┌─────────┴───────┐          ┌───────────┐
  │   acid    │..........│      core       │..........│   acid    │
  │   group   │          │    molecule     │          │   group   │
  └───────────┘(optional)└─────────────────┘(optional)└───────────┘
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wherein the core molecule has a conformation and polarity such that the acid group(s) interact with one of more of His6, His13 and His14. The acid may be any acid group, including, but not limited to, CO_2H, PO_3H_2, SO_3H, OSO_3H_2, OPO_3H_2 and the like.

The compound may be a molecule with one to three carboxylic acid groups, the length of the molecule being such that it can be received within the N-terminal loop, and such that at least one carboxyl group is in proximity to at least one of the histidine residues. Without wishing to be bound by theory, we believe that it is likely that such molecules will have a molecular mass in the region of 2000 Daltons.

The compound may be an organic molecule, a peptide or a metal complex. In this aspect of the invention, however, it is preferred that the compound is not a metal complex. Preferably the compound has overall hydrophobic character. More preferably the compound is able to penetrate the blood-brain barrier.

In a particularly preferred embodiment of the invention, the inhibitor compound comprises, or is conjugated to, a targeting moiety.

The term "targeting moiety" as used herein refers to a functional group which will specifically interact with the β-amyloid peptide. That is, the inhibitor compound includes or is covalently linked to a targeting moiety which will specifically bind to or associate with the β-amyloid peptide. Suitable targeting moieties include,

but are not limited to, polypeptides, nucleic acids, carbohydrates, lipids, β-amyloid ligands, antibodies, dyes and the like. In a preferred embodiment the targeting moiety has a hydrophobic region which interacts with the

5   tail of the β-amyloid peptide. For example, the targeting moiety may include a fatty acid molecule.

Preferably the targeting moiety targets the compound to the site defined by residues 15-21 of the β-amyloid peptide. The targeting moiety may be a peptide which

10 comprises a sequence which corresponds to that of residues 15-21 of the β-amyloid peptide. More preferably the inhibitor-targeting moiety complex is able to penetrate the blood-brain barrier.

In a second aspect, the invention provides a method

15 of selecting or designing a compound which inhibits the binding of metal ions to the N-terminal loop of the β-amyloid peptide, which method comprises the steps of

(i) selecting or designing a compound which has a conformation and polarity such that it binds to at least

20 one, more preferably at least two and more preferably three amino acids in the N-terminal loop, selected from the group consisting of His6, His 13 and His14; and

(ii) testing the compound for the ability to inhibit binding of metal ions to the N-terminal loop of the

25 β-amyloid peptide.

Preferably the compound inhibits binding of Cu^{2+}, Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

Preferably the compound has a conformation or polarity such that it also binds to at least one amino acid

30 in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11. Preferably the compound also has overall hydrophobic character. More preferably the compound is able to penetrate the blood-brain barrier.

In a third aspect, the invention provides a compound

35 which inhibits the binding of metal ions to the N-terminal loop of the β-amyloid peptide, wherein the compound is

obtained by a method according to the second aspect of the invention.

In a fourth aspect, the invention provides a composition comprising a compound according to the first or
5   the third aspects of the present invention, together with a pharmaceutically acceptable carrier. Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 19th
10   Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

The compounds of the present invention may be formulated into pharmaceutical compositions, and administered in therapeutically effective doses. The term
15   "therapeutically effective dose" means a dose which results in the inhibition of natural binding of metal ions to the N-terminal loop of the β-amyloid peptide. The pharmaceutical compositions may be administered in a number of ways, including, but not limited to, orally,
20   subcutaneously, intravenously, intraperitoneally and intranasally. The most appropriate dose and route of administration will be dependent on the age and general state of health of the subject to be treated, and will be at the discretion of the attending physician. This dose can
25   be readily ascertained by one skilled in the art, using well-known techniques.

In a fifth aspect, the invention provides a method of inhibiting the binding of one or more metal ions to the β-amyloid peptide, or inhibiting the aggregation of β-
30   amyloid peptide, which method comprises the step of exposing the peptide to a compound which blocks or destabilises the N-terminal loop of the peptide, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

35   Preferably the compound has a conformation and polarity such that it binds to at least one, more preferably at least two, and more preferably three

histidine residues in the N-terminal loop of the β-amyloid peptide, selected from the group consisting of His6, His13 and His14. More preferably the compound also binds to at least one additional amino acid in the N-terminal loop,

5 selected from the group consisting of Asp7, Tyr10, and Glu11.

In a particularly preferred embodiment, the compound is a metal complex which can exchange or bind functional moieties such as histidine, with the proviso that the

10 compound is not haemin or haematin. Preferably the metal complex is capable of binding between 1 and 3, preferably 2 or 3, histidine residues of the N-terminal loop of the β-amyloid peptide. The complex may bind to other residues in addition to the histidine residues. More preferably the

15 complex also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

Metal ions capable of binding to the imidazole nitrogen(s) of histidine include Mn, Fe, Co, Ni, Cu, Zn,

20 Ru, Pd, Ag, Cd, Pt, Au, Rh and Hg. Complexes of these metals are expected to be predominantly four coordinate tetrahedral (distorted tetrahedral)/square planar) complexes, five coordinate complexes with either a trigonal bipyramid or square pyramid configuration, or six

25 coordinate octahedral (or distorted octahedral) complexes.

Even more preferably the inhibitor compound comprises, or is conjugated to, a targeting moiety. Preferably the targeting moiety targets the compound to the site defined by residues 15-21 on the β-amyloid peptide.

30 In a further preferred embodiment, the inhibition of binding of one or more metal ions to the β-amyloid peptide occurs *in vivo*.

In a sixth aspect, the invention provides a method of prevention, treatment or alleviation of Alzhemier's disease

35 which method comprises the step of administering a compound or a pharmaceutical composition according to the invention to a subject in need of such treatment.

The patient is monitored for clinical improvement, which may commence within as little as one week, but more probably may be observed at six weeks, and may take as long as 12 months. The normal clinical indices which are used in
5   the monitoring of patients with the relevant condition are used. Where the treatment is prophylactic, the patient is monitored for signs of development of the condition. The attending clinician will be aware of the most suitable tests to use.

10   Where this method is to be used for prophylactic purposes, the subject is preferably one at increased risk of developing the condition. For example, the subject may have one or more family members with the condition, eg. familial Alzheimer's disease, or may have trisomy of
15   chromosome 21 (Down's syndrome).

It will be clearly understood that, for the purposes of this aspect of the invention, the compound is not haemin.

In a seventh aspect, the invention provides a method
20   of monitoring the efficacy of treatment according to the method of the invention, comprising the steps of obtaining a sample of a biological fluid for a patient undergoing treatment, and measuring the level of Aβ in the sample, in which increased Aβ levels compared to levels of Aβ in a
25   normal control sample are indicative of the efficacy of the treatment.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word
30   "comprises" has a corresponding meaning.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a representation of the β-amyloid peptide showing a structured turn in the region of amino acid
35   residues 15-21.

Figure 2 shows a model of Zn bound to the three histidine residues of Aβ1-40.

Figure 3 is a NMR spectrum showing the effect of Zn^{2+} binding to Aβ1-28.

Figure 4 is a NMR spectrum showing the effect of Cu^{2+} binding to Aβ1-28.

Figure 5 is a NMR spectrum showing the effect of addition of Cu^{2+} and cobalt complex to Aβ1-28.

Figure 6 is a NMR spectrum showing the binding of the cobalt complex to Aβ1-28.

Figure 7 is a Western blot showing results of brain tissue assays testing the ability of a range of metal compounds to solublilize Aβ deposits.

Figure 8 shows a model of the cobalt-corrin ring complex bound to Aβ1-40.

Figure 9 shows NMR spectra demonstrating the binding of the compound KJB001 to Aβ1-28.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have developed three-dimensional structural information concerning the N-terminal region of the β-amyloid peptide, and have identified a cluster of three histidine residues which constitute a binding site for metal ions. This information provides a rational basis for the development of compounds which inhibit the binding of metal ions to the N-terminal loop of the β-amyloid peptide. Such inhibitors have the potential to inhibit aggregation of β-amyloid peptides and to reduce metal- induced neurotoxicity. Accordingly, these inhibitors are likely to have therapeutic value in the treatment of diseases such as Alzheimer's disease.

Using the structural information provided by the inventors, the general principles of drug design can be applied by persons skilled in the art to produce compounds which preferentially bind to at least one of the histidine residues in the N-terminal loop (ie His6, His13 or His14), and inhibit the binding of metal ions to the N-terminal loop of the amyloid peptide.

Preferred inhibitors within the context of the present invention include metal complexes which can exchange or bind functional moieties such as histidine. Preferably the metal complex is capable of binding between 1 and 3, preferably 2 or 3, histidine residues of the N-terminal loop of the β-amyloid peptide. The complex may bind to other residues in addition to the histidine residues. Metal ions capable of binding to the imidazole nitrogen of histidine include Mn, Fe, Co, Ni, Cu, Zn, Ru, Pd, Ag, Cd, Pt, Au, Rh and Hg . Complexes of these metals are expected to be predominantly four coordinate tetrahedral (distorted tetrahedral)/square planar complexes, or six coordinate octahedral (or distorted octahedral) complexes. In the case of the four coordinate complexes they could react with the β-amyloid peptide to replace ligands; alternatively, by binding to His residues as well as to their initial ligand(s) their coordination number is increased to 5 or 6. Five coordinate complexes with either a trigonal bipyramid or square pyramid configuration may also be used. Examples of suitable complexes are shown below.
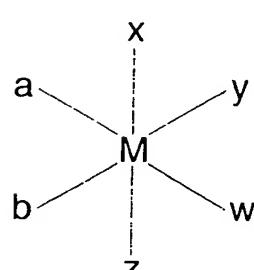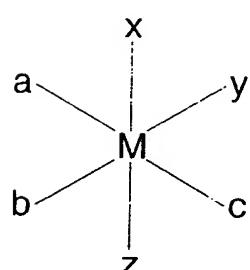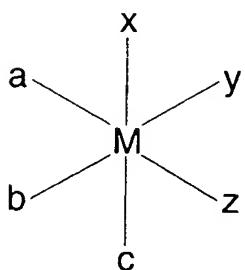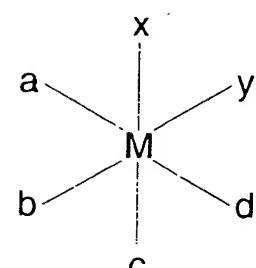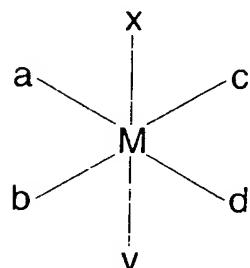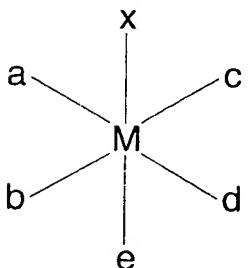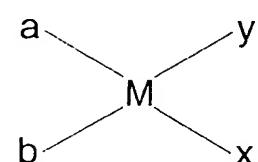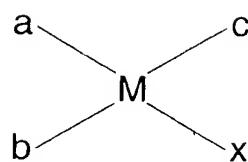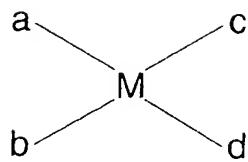
In these formulae:

    (i)   a, b, c, d and e are non-leaving groups,
preferably chelating groups including Schiff bases,
porphyrin rings, macrocycles, polyamino-carboxylates,
heterocyclic aromatic groups such as 2,2'bipyridine and
1,10-phenanthrolene, peptides, nucleobases, or chelating
ligands in which one of the donor atoms is a phosphine
phosphorus atom;

    (ii)   M is a metal; and

    (iii) w, x, y and z are leaving groups (ie those
groups which will be replaced by histidine and possibly
other residues when the metal complex reacts with the
β-amyloid peptide), and include halogens, amines, ammonia,
pyridyls, imidazoles, nucleobases, peptides,
H_2O/OH, carboxylic acids, phosphates, sulfates, nitrate,
triflate, or alkoxides.

The term " nucleobase" means a purine or a
pyrimidine, or an analogue thereof. It will be appreciated
that multidentate macrocyclic ligands may have a variety of
donor atoms, and that it is possible that one or more of
the non-leaving groups could be a stable monodentate ligand
such as cyanide, or an organic group such as a methyl
group.

Those skilled in the art will recognise that the
appropriate combination of non-leaving and leaving groups
will be dependent on the identity of the metal.

Metal complexes which have the potential to bind to
histidine residues are described in WO 97/21431 and WO
96/18402, the entire contents of which are incorporated
herein by this reference. Other examples of complexes which
may act as inhibitors are as follows:

I

II

III
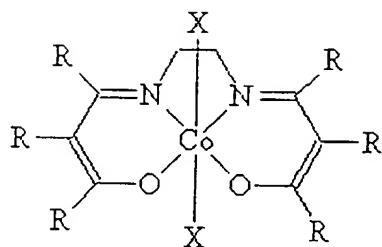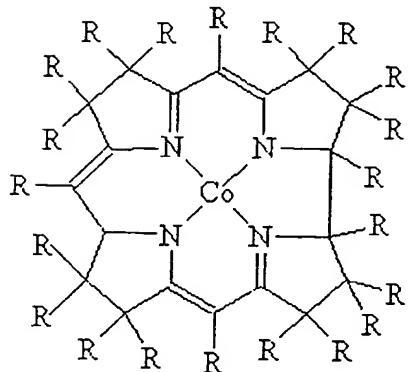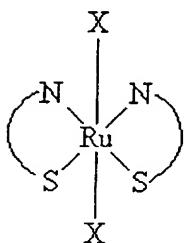
In formulae I, II and III, the X and R groups may be any suitable leaving or targeting groups. For example, X and R may be the same or different, and include, without being limited to, the group consisting of ammine; amine; peptide; halogen (chloride, fluoride or iodide); nucleobase; imidazole; H_2O; hydrogen; saturated or unsaturated alkyl, alcohol, or carboxylate of 1 to 10 carbon atoms; aromatic; or heterocyle of up to four rings.

Other preferred compounds include cobalt(I1) phthalocyanine β-form; (S,S)-(+)-N-N'-bis(3,5-di-tert-butylsalicylidene)-1-2-cyclohexane diamino manganese (III)

chloride; iron(II) phthalocyanine bis(pyridine) complex; iron(III) phthalocyanine chloride; manganese(II) phthalocyanine; 5,10,15,20-tetraphenyl-21H, 23H-porphine manganese (III) chloride; chloro(pyridine) bis

5   (dimethylglyoximato) cobalt (III); N-N'-bis(salicylidene)dianilino-cobalt(II);cis-bis(2-2'-bipyridine)dichloro-ruthenium (II) hydrate; and cobalt(acacen)$(NH_3)_2)Cl$, in which (H_2acacen represents bis(acetylacetone)-ethylenediamine).

10   Compounds which are designed or selected according to the methods of the invention may be tested for inhibitory activity by any suitable assay procedure. Assays to determine the binding of metal complexes to Aβ may be performed by NMR or UV-Visible spectroscopy, or by ESR in

15   the case of paramagnetic metals. Assays are available for measuring Cu/Fe reduction, hydrogen peroxide, hydroxyl radical generation, and carbonyl group, all of which assess the redox capacity of Aβ in the presence of Cu and Fe. *Ex vivo* assays using *post mortem* brain tissue may also be

20   performed. These include measuring the amount of Aβ which is solubilised and extracted in the presence of the compound, and determining the quantity of peroxide formed in *post mortem* brain tissue, as compared with control tissue which is solubilised and extracted in the absence of

25   the compound. Suitable methods are described for example in PCT/US99/05291 (WO99/45907).

         The invention will now be described in detail by way of reference only to the following non-limiting examples and to the drawings.

30

Materials and Methods

NMR spectroscopy

Samples of Aβ1-40 and Aβ1-28 in aqueous solution were
prepared by dissolving 0.5mgs and 1.0 mgs respectively in

5   0.55 ml of 10 % ^{2}H20/90%H$_2$O, 100 mM KCl and 50 mM phosphate
buffer. pH values were measured at room temperature, and
were not corrected for isotope or solvent effects. The 1H
chemical shifts were referenced to 2,2-
dimethyl-2-silapentane-5-sulphonate at 0 ppm, via the

10   chemical shift of the H$_2$O resonance (Wishart et al., 1995a)
or an impurity at 0.15 ppm.

Spectra were recorded on a Bruker DRX-600
spectrometer. All spectra in aqueous solution were recorded
at 271 K and pH 6.8, with probe temperatures calibrated

15   according to the method of van Geet (1970). All 2D spectra
were recorded in phase-sensitive mode using
time-proportional phase incrementation (Marion & Wuthrich,
1983). Water suppression was achieved using pulsed field
gradients with the WATERGATE scheme and a 3-9-19 selective

20   pulse (Sklenar et al., 1993).

2D homonuclear NOESY spectra (Anil-Kumar et al.,
1980; Macura et al., 1981) were recorded with mixing times
of 50 and 250 ms. TOCSY spectra (Braunschweiler & Ernst,
1983) were recorded using the DIPSI-2 spin-lock sequence

25   (Rucker & Shaka, 1989) with spin-lock times of 70-80 ms.
DQF- COSY (Rance et al., 1983) spectra were also recorded.
Typically, spectra were acquired with 400-600 t_1
increments, 32-128 scans per increment, and 4096 data
points. The ^{1}H sweep width was 7575.6 Hz at 600 MHz.

30   Spectra were processed using UXNMR-941001.4 (Bruker) and
analyzed using XEASY 1.3.7 (Bartels et al., 1995).
Sine-squared window functions, phase shifted by 60°-90°,
were applied in both dimensions prior to Fourier
transformation.

35   The $^3J_{NHC\alpha H}$ coupling constants were measured from a
DQF-COSY spectrum or by using the INFIT module of XEASY to
analyze NOESY spectra.

For $^3J_{NHC\alpha H}$ coupling constants measured from a DQF-COSY the appropriate rows were extracted from the spectrum, inverse Fourier transformed, zero filled to 32 K data points, and multiplied by a Gaussian window function prior
5   to Fourier transformation. The dispersive peak shapes were simulated to take account of the effect of broad linewidths on small coupling constants, using an in-house program, COUPLING.

10 *Metal Binding Studies*

Metal binding studies were performed by titrating concentrated metal solutions (30 mM $CuCl_2$, $ZnCl_2$ in water) into the peptide solutions described above. The displacement of bound Cu^{2+} from Aβ1-28 by the Co(III)
15 Schiff-base was performed by adding two equivalents of Cu^{2+} to Aβ1-28, followed by one equivalent of Co(III) Schiff-base.

Brain Tissue Assays
20 *Tissue selection*

Post-mortem tissues, stored at -80 °C, were obtained from the NH&MRC-supported Brain Bank at the University of Melbourne, together with accompanying histopathological and clinical data. AD was assessed according to CERAD criteria
25 (Mirra et al, 1991). In order to examine the chemical architecture of the Aβ deposition observed in non-AD aged brain, Aβ immunohistochemistry was used to select age-matched control (AC) cases which did not fulfil the CERAD criteria, and in which amyloid deposition, if
30 present, was detectable only in the form of diffuse plaques, but not neuritic plaques.

Preparation of compounds

Compounds were dissolved in DMSO and diluted in a PBS
35 mixture. Insoluble material was removed.

Sample preparation:

The cortical meninges were removed and gray matter
(0.5 g) was homogenised using a DIAX 900 homogeniser
(Heidolph & Co, Kelheim, Germany) for 3 x 30s periods at
full speed, with a 30s rest between strokes, in 3 ml of
5   ice-cold phosphate-buffered saline (PBS), pH 7.4,
containing a mixture of protease inhibitors (BioRad,
Hercules, CA), but without ethylene diamine tetraacetic
acid (EDTA), or in the presence of test compounds or metal
ions prepared in PBS. The homogenate samples were
10   incubated for 24 h at room temperature. To obtain the
PBS-extractable fraction, the homogenate was centrifuged at
100,000 x g for 30 min, the supernatant removed, and
divided into 1 ml aliquots. Protein in a 1 ml supernatant
sample was precipitated using 1:5 ice-cold 10%
15   trichloracetic acid (TCA), and pelleted by centrifugation
at 10,000 x g for 20 mins. The pellet was prepared for PAGE
by boiling for 10 min in Tris-tricine SDS-sample buffer
containing 8% SDS, 10% mercaptoethanol and 8M urea. Total
Aβ in the cortical samples was obtained by homogenizing in
20   1 ml PBS and boiling in sample buffer as described above.

*Polyacrylamide gel electrophoresis (PAGE) and Western
blotting*

Tris-tricine PAGE was performed by loading samples on
25   to 10-20% gradient gels (Novex, San Diego, CA), followed by
transfer on to 0.2 mm nitrocellulose membrane (BioRad,
Hercules, CA). The Aβ was detected using the following
monoclonal antibodies: W02, which detects Aβ40 and Aβ42 at
an epitope between residues 5 and 8; G210, which is
30   specific for Aβ species which terminate at carboxyl residue
40; or G211, which is specific for Aβ species which
terminate at carboxyl residue 42 (Ida et al, 1996), in
conjunction with horseradish peroxidase(HRP)-conjugated
rabbit anti-mouse IgG (Dako, Denmark), and visualised using
35   chemiluminescence (ECL, Amersham Life Science, UK). Each
gel included two or more lanes containing known quantities
of synthetic Aβ (Keck Laboratory, Yale University New

Haven, CT) as reference standards. Blot films were scanned using a Relisys scanner with transparency adapter (Tech Information Systems, Taiwan) and densitometry performed using Image 1.6 software (NIH, Bethesda, MA). All samples were analysed at least twice, and gel loadings and dilutions adjusted to fit within the quantifiable region of the standard curve.

The efficiency of the TCA precipitation procedure was validated by testing samples of whole human serum diluted 1:10 to which had been added 2mg of synthetic Aβ 1-40 or Aβ 1-42. Aβ recovery was assessed by extracting the precipitate into SDS sample buffer and performing Western blot analysis, using synthetic Aβ standards as described above. Protein in the TCA pellet was estimated by resuspending the pellet in water and assaying the protein recovery using a BCA assay (Pierce, Rockford, IL). This indicated that the efficiency of protein and Aβ precipitation was approximately 90%. The efficiency of the 8M urea solubilization was found to be equivalent to that of formic acid in a parallel, blinded assay conducted independently. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Example 1   Characterisation of Aβ peptides in aqueous solution

In aqueous solution there is little chemical shift difference between the amide and $C^{\alpha}H$ protons of Aβ1-28 compared with Aβ1-40, suggesting that both peptides are in a similar conformation. Comparisons of A1-28 and Aβ1-40 chemical shifts with random coil chemical shifts and the lack of NOE connectivities in the NOESY spectra indicate that both peptides are mostly in conformational exchange. However, there are some medium range NOE connectivities (1 < /ii-j/ < 5) observed in the region of residues 16-21 of the peptide (KLVFFA), suggesting that this region of the peptide has a structured turn. This is illustrated in Figure 1.

This region of the peptide has previously been shown
to be very important in defining the aggregation properties
of Aβ (Hilbich et al. 1992), with the substitution of
hydrophilic residues into this region resulting in altered
5   aggregation properties, including reduced β-sheet content.
In addition, several groups have described short peptides
or slight variants thereof corresponding to this region
which have the ability to bind to Aβ and to inhibit the
formation of amyloid fibrils (Findeis et al. 1999;
10   Tjernberg et al. 1999). This evidence implies that this
"structured" section of Aβ is important in the formation of
amyloid fibrils.

Example 2   **Metal Binding Studies**

15   To determine the metal-binding site of Aβ1-40, Zn^{2-}
was titrated into a solution of Aβ1-40 in SDS-micelles at
pH 6.5. Peaks due to the C2H protons of the imidazole rings
of His6, His13 and His14 broadened out such that they were
no longer visible when a small amount of Zn solution (~ 25%
20   of one mol. equivalents) was added. The addition of extra
Zn (up to two mol. equivalents) did not change the
spectrum, but when the pH of the solution was raised to 7.4
three broad overlapping peaks due to the C2H protons of the
imidazole rings of His6, His13 and His14 became visible.
25   These peaks did not sharpen significantly even upon the
addition of a large excess of Zn (> 150 mol. equivalents).
There appear to be no significant difference in the rest of
the spectrum between the Zn-bound and free forms of Aβ1-40,
suggesting that there are no significant conformational
30   changes upon metal binding. These results indicate that all
three histidine residues of Aβ1-40 are involved in Zn
binding. Figure 2 shows a model of Zn bound to the three
histidine residues of Aβ1-40.

To determine the metal-binding site of Aβ1-40 and
35   Aβ1-28 in aqueous solution, Zn^{2+} and Cu^{2+} were titrated into
solutions of Aβ1-40 and Aβ1-28 at pH 6.9. All reactions
were accompanied by significant precipitation. The NMR

spectrum of the peptide-metal complex which remained in
solution showed that peaks due to the C2H and C4H protons
of His6, His13 and His14 broadened out such that they were
no longer visible, indicating that these residues were
5   involved in metal binding. This is illustrated in Figures 3
and 4. The addition of more metal ion resulted in more
precipitation, so that saturated binding was not possible.
        When the Co(III) Schiff-base complex was added to a
solution containing Cu^{2+}-bound Aβ1-28, a broad peak
10 appeared in the 1H spectrum at 6.55 ppm, as shown in Figure
5. The chemical shift of this peak is consistent with the
chemical shift of a C4H proton of a histidine imidazole
bound to a Co(III)Schiff-base complex, as shown in Figure
6. This indicates that the Co(III)Schiff-base complex can
15 compete with Cu^{2+} for the histidine residues of Aβ.

Example 3   **Brain tissue assays**
        Brain tissue assays were conducted in order to test
the ability of the following compounds to reduce β-amyloid
20 peptide aggregation:

KJB001	Co(II) phthalocyanine β-form
KJB002	(S,S)-(+)-N-N'-bis(3,5-di-tert-butylsalicylidene)-1-2-cyclohexane diamino manganese (III) chloride
KJB003	Haemin
KJB004	Iron(II) phthalocyanine bis(pyridine) complex
KJB005	Iron(III) phthalocyanine chloride
KJB006	Manganese(II) phthalocyanine
KJB007	5,10,15,20-tetraphenyl-21H,23H-porphine manganese(III) chloride
KJB008	Chloro(pyridine) bis(dimethylglyoximato)cobalt (III)
KJB009	N-N'-bis(salicylidene)dianilino-cobalt(II)
KJB010	cis-bis(2-2'-bipyridine)dichloro-ruthenium (II) hydrate.

BR16805   [Co(acacen)(NH3)$_2$]Cl (H$_2$acacen represents
           bis(acetylacetone)ethylenediamine)

5   Table 1 shows the approximate concentration of each
   compound used in the extraction.

Table 1

Compound	Concentration (μmol/litre)
KJB001	0.0105
KJB002	4.41
KJB003	60.87
KJB004	3.816
KJB005	3.08
KJB006	111.5
KJB007	0.64
KJB008	1289.9
KJB010	300.9
BRI6805	1199.9
bathocuproine	2000

10   The results presented in Figure 7 clearly demonstrate
    that some of the metal complexes have the ability to
    solubilise Aβ deposited in the brains of Alzheimer's
    disease patients. In particular, compound KJB001 showed
    particularly strong solubilising activity, and compounds
15 KJB002, KJB005, KJB006, KJB007 and KJB010 also showed good
    activity.

Example 4   **Compounds designed to bind one or more of**
                     the three histidine residues in Aβ
20   BRI7080, BRI7103 and BRI7104 are metal complexes of
   aza-macrocycles of the following structures.

(a)   BRI7080
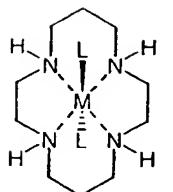
BRI7080 : M = Ni^{2+} L = H$_2$O

Ni(NO$_3$)$_2$.6H$_2$O (1.67 g, 5.75 mmol) was added in portions to a
solution of 1,4,8,11-tetraazacyclotetradecane (1.0 g, 5.00
5   mmol) in ethanol (50 ml) at 50 °C. The mixture was heated
at 50 °C for 2 h during which time the green coloured
nickel salt dissolved to give a red solution. Upon cooling
of the crude reaction mixture to room temperature, a purple
solid had precipitated which was collected by filtration
10   and washed with several portions of 2-propanol. The
product thus obtained was dried overnight in a vacuum oven.
Spectral and analytical data acquired for this compound
were in agreement with literature data.

15   (b)   BRI7103
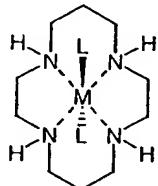
BRI7103 : M = Mn^{3+} L = Cl

BRI7103 was prepared following the method of P-K. Chan and
C-K. Poon, *J. C. S. Dalton Trans.*, 1976, 858-862.

20   (c)   BRI7104

This compound has similar structures to BRI7080 and
BRI7103, but in this case M = Co^{3+} and L = Cl.

BRI7104 was prepared following the method of P-K. Chan and
C-K. Poon, *J. C. S. Dalton Trans.*, 1976, 858-862.

Example 5   **Histidine binding compounds conjugated with**
5   **an Aβ-directing group**.

For the second class of molecules we chose
derivatives of the pentapeptide leucine-valine-
phenylalanine-phenylalanine-alanine (LVFFA), designated
herein as BRI7082 and BRI7077, to direct histidine binding
10   compounds to Aβ.

· BRI7106 is an example of a nicotine derivative
conjugated to an Aβ-directing group. In this example the
histidine-binding group is a small organic molecule rather
than a metal complex, in contrast to most of the compounds
15   described above. BRI7158 and BRI7159 are examples of metal
complexes conjugated to an Aβ-directing group.


(a)   BRI7082
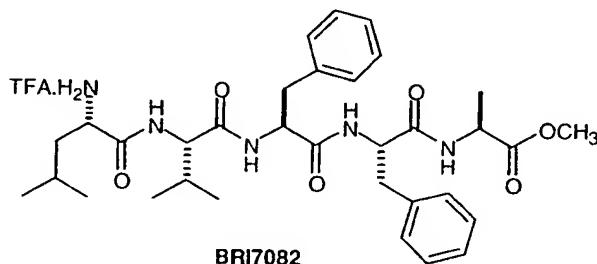
BRI7082

20   BRI7082 was prepared *via* standard HBTU-mediated peptide
coupling methodology; see M. Bodanszky and A. Bodanszky,
"The Practice of Peptide Synthesis", 2nd Edition, Springer-
Verlag, 1994.
Mass Spectrum (APCI) *m/z* 610 [(MH-$C_2HO_2F_3$)$^+$, 100%].
25

(b)   BRI7106
This compound was synthesised according to Reaction Scheme
1:
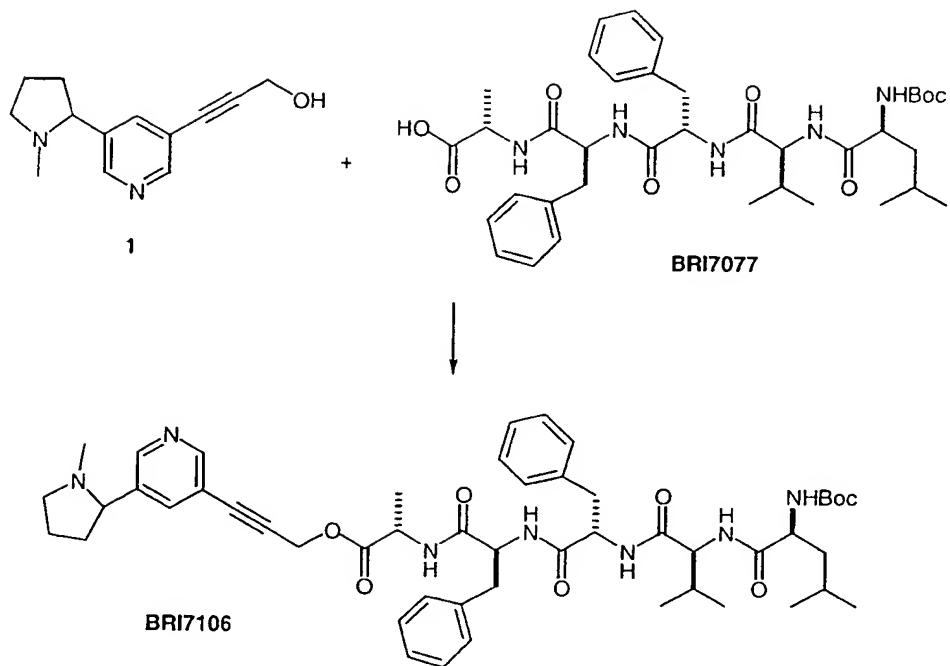
Scheme 1

    Compound 1 was prepared following the methods of P.
Jacob III, *J. Org. Chem.*, 1982, 47, 4165-4167 and N. D. P.
Cosford *et al.*, *J. Org. Chem.*, 1998, 63, 1109-1118.

5 Mass Spectrum (APCI) *m/z* 217 [(M+H)$^+$, 100%].

    BRI7077 was prepared *via* standard HBTU-mediated
peptide coupling methodology; see M. Bodanszky and A.
Bodanszky, "*The Practice of Peptide Synthesis*", 2nd
Edition, Springer-Verlag, 1994.

10 Mass Spectrum (APCI) *m/z* 696 [(M+H)$^+$, 20%], 694 [(M-H),
95%].

    BRI7106 was prepared *via* standard DCC-mediated
esterification methodology; see M. Bodanszky and A.
Bodanszky, "*The Practice of Peptide Synthesis*", 2nd

15 Edition, Springer-Verlag, 1994.

Mass Spectrum (APCI) *m/z* 894 [(M+H)$^+$, 10%], 892 [(M-H),
25%].

(c)   Synthesis of BRI7158

This compound was synthesised according to Reaction Scheme
2:

3a : R = CH₃
3b : R = H

BRI7158
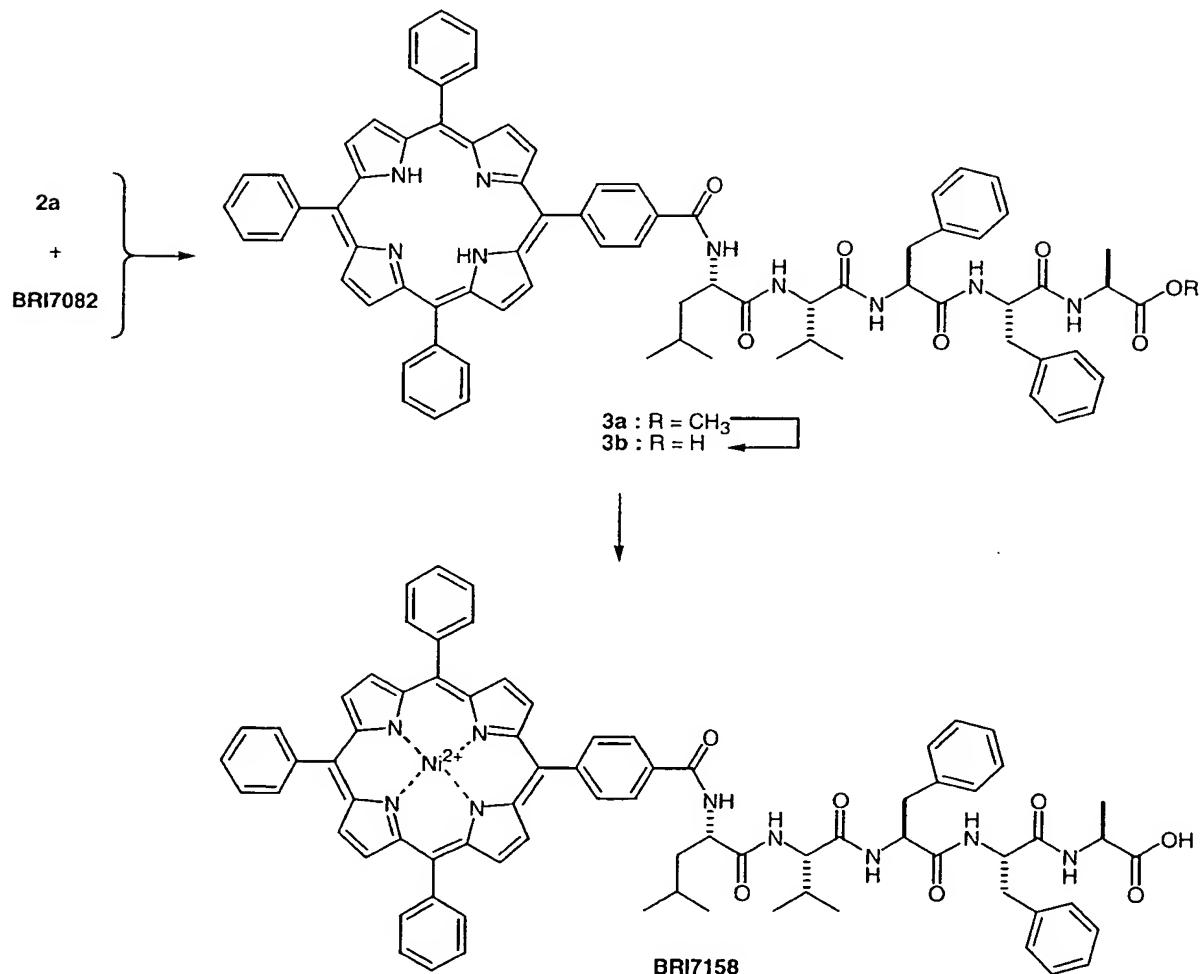
Scheme 2

5

Porphyrin 2a was prepared following the methods of K.
Nakanishi *et al.*, *Heterocycles*, 1996, 42, 723-736 and D. A.
James *et al.*, *Bioorg. Med. Chem. Lett.*, 1999, 9, 2379-2384.
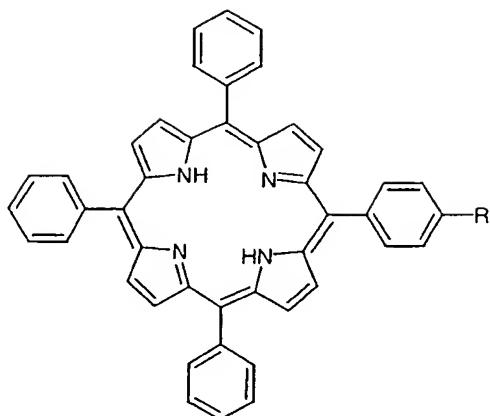
2a : R = CO₂H

  N, N-Diisopropylethylamine (0.10 ml, 0.57 mmol) was
added to a magnetically stirred mixture of porphyrin 2a
(118 mg, 0.18 mmol), HBTU (68 mg, 0.18 mmol), and BRI7082
5 (130 mg, 0.18 mmol) in dry DMF (4 ml) under an atmosphere
of nitrogen. Stirring was continued at room temperature
for 16 h, after which time, the crude reaction mixture was
partitioned between ethyl acetate and brine. The separated
organic layer was successively washed with 10% aqueous
10 citric acid solution, saturated aqueous NaHCO₃ solution and
brine before being dried (MgSO₄), filtered and concentrated
under reduced pressure to afford compound 3a as a deep
purple, crystalline solid.
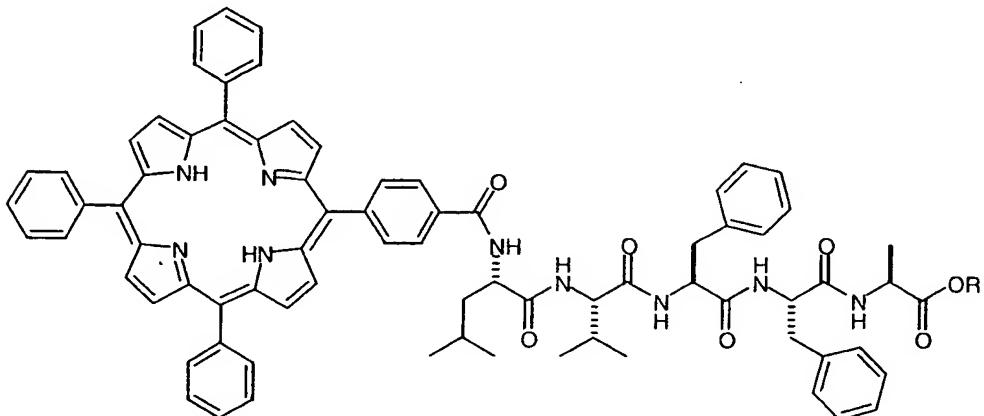
3a : R = CH₃

Mass Spectrum (APCI) m/z 1250 [(M+H)$^+$, 45%].

A solution of compound 3a (23.3 mg, 0.019 mmol, THF (0.5 ml), methanol (50 drops) and 2M aqueous NaOH solution (0.1 ml) was stirred at room temperature for 18 h. The crude reaction mixture was partitioned between ethyl acetate and water and the phases separated. The aqueous layer was acidified to pH 4 upon the addition of 10% aqueous citric acid solution and then extracted three times with ethyl acetate. The combined organic layers were dried (MgSO$_4$), filtered and concentrated under reduced pressure to afford compound 3b as a purple solid.
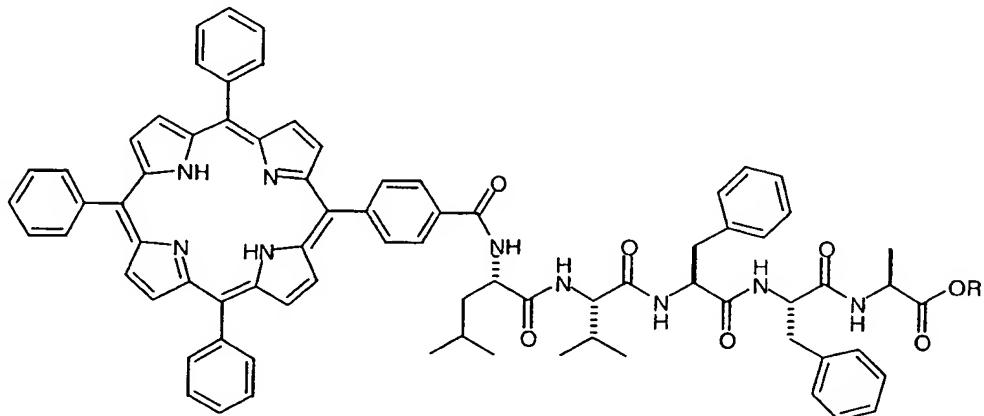

3b : R = H

Mass Spectrum (APCI) m/z 1236 [(M+H)$^+$, 30%].

A magnetically stirred mixture of compound 3b (17 mg, 0.014 mmol), Ni(OAc)$_2$.4H$_2$O (34.2 mg, 0.14 mmol), glacial acetic acid (0.73 ml) and dichloromethane (1.45 ml) was heated at reflux under an atmosphere of nitrogen for 18 h. The now crimson red reaction mixture was cooled to room temperature and partitioned between ethyl acetate and 10% aqueous NaHCO$_3$ solution. The separated aqueous phase was extracted three times with ethyl acetate before being dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give a red solid. Subjection of this material to flash chromatography (silica, 10% methanol/dichloromethane

elution) afforded, after concentration of the appropriate fractions (R_f 0.30), BRI7158 as a red, crystalline solid. Mass Spectrum (ES) m/z 1291 [(M-H), <10%].

5   (d)   Synthesis of BRI7105 and BRI7159

These compounds were synthesised according to Reaction Scheme 3:
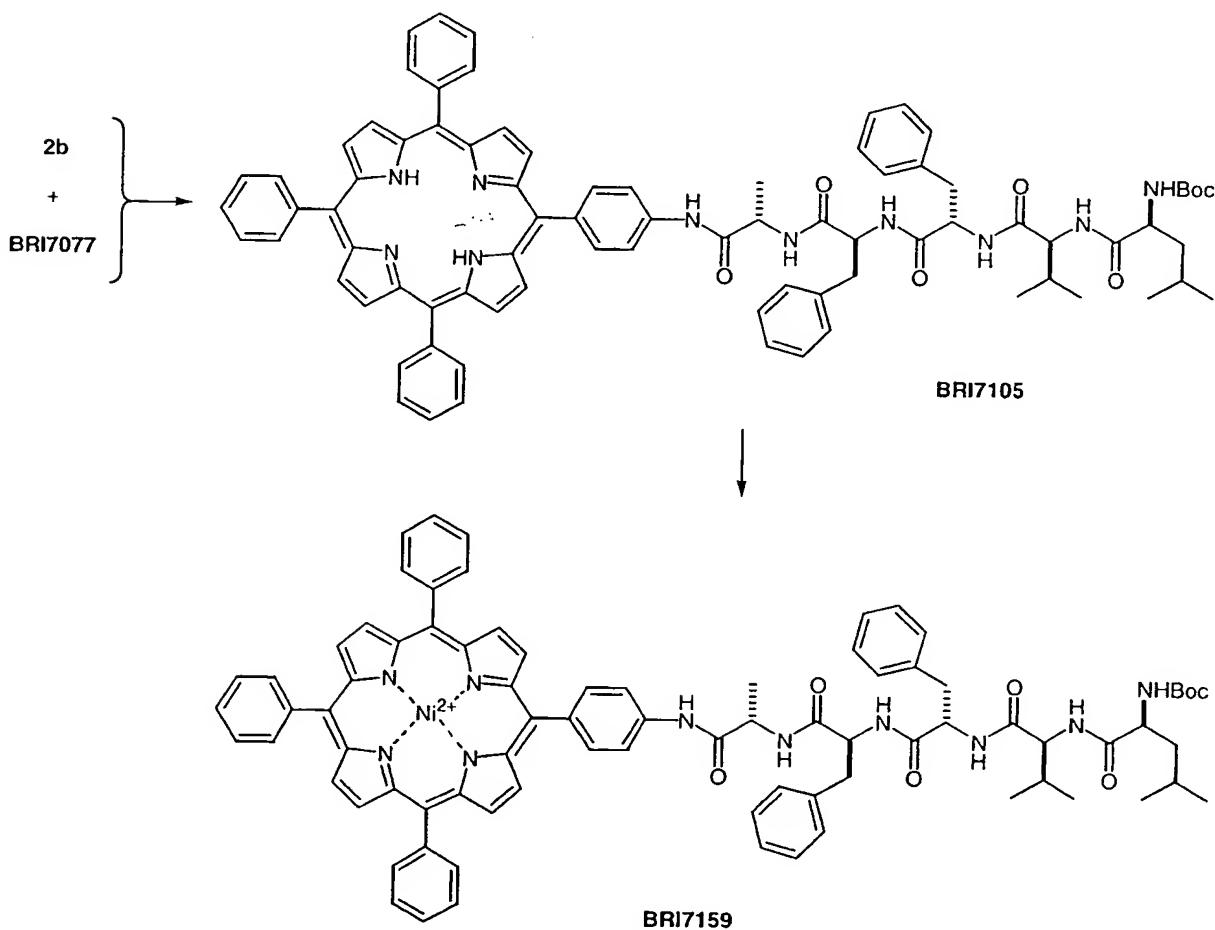
Scheme 3

Porphyrin 2b was prepared following the method of W. J. Kruper, Jr. *et al.*, *J. Org. Chem.*, 1989, 54, 2753-2756.
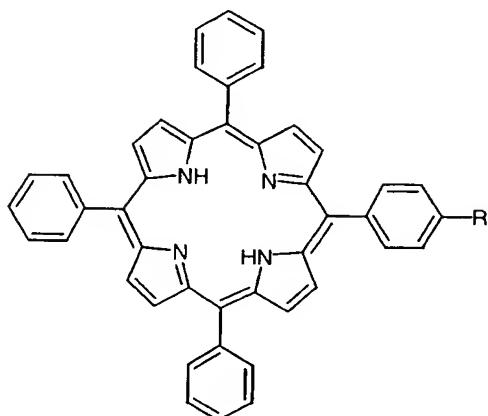
2b : R = NH$_2$

N, N-Diisopropylethylamine (0.21 ml, 1.18 mmol) was
5 added at room temperature to a magnetically stirred mixture
of porphyrin 2b (151 mg, 0.24 mmol), HBTU (216 mg, 0.58
mmol), and BRI7077 (200 mg, 0.29 mmol) in dry DMF (2 ml)
under an atmosphere of nitrogen. Stirring was continued at
40°C for 40 h, after which time the crude reaction mixture
10 was partitioned between ethyl acetate and brine. The
separated organic layer was successively washed with 10%
aqueous citric acid solution, 5% aqueous NaHCO$_3$ solution
and brine before being dried (MgSO$_4$), filtered and
concentrated under reduced pressure to afford a deep purple
15 glass. Subjection of this material to flash chromatography
(silica, 1% to 5% methanol/dichloromethane elution) gave,
after concentration of the relevant fractions (R$_f$ 0.35),
BRI7105 as a purple, crystalline solid.
Mass Spectrum (APCI) *m/z* 1307 [(M+H)$^+$, <5%].
20   A magnetically stirred mixture of BRI7105 (47 mg,
0.04 mmol), Ni(OAc)$_2$.4H$_2$O (96 mg, 0.39 mmol), glacial
acetic acid (2 ml) and dichloromethane (4 ml) was heated at
reflux under an atmosphere of nitrogen for 18 h. The now
crimson red reaction mixture was cooled to room temperature
25 and partitioned between ethyl acetate and 10% aqueous

NaHCO₃ solution. The separated aqueous phase was extracted three times with ethyl acetate before being dried (Na₂SO₄), filtered and concentrated under reduced pressure to give a red solid. Subjection of this material to flash

5   chromatography (silica, 10% methanol/dichloromethane elution) afforded, after concentration of the appropriate fractions (R$_f$ 0.30), BRI7159 as a red, crystalline solid. Mass Spectrum (APCI) *m/z* 1385 [(M+Na)⁺, <5%].

10   (e)   Synthesis of BRI7160 and BRI7161

The compounds were prepared according to Reaction Scheme 4:
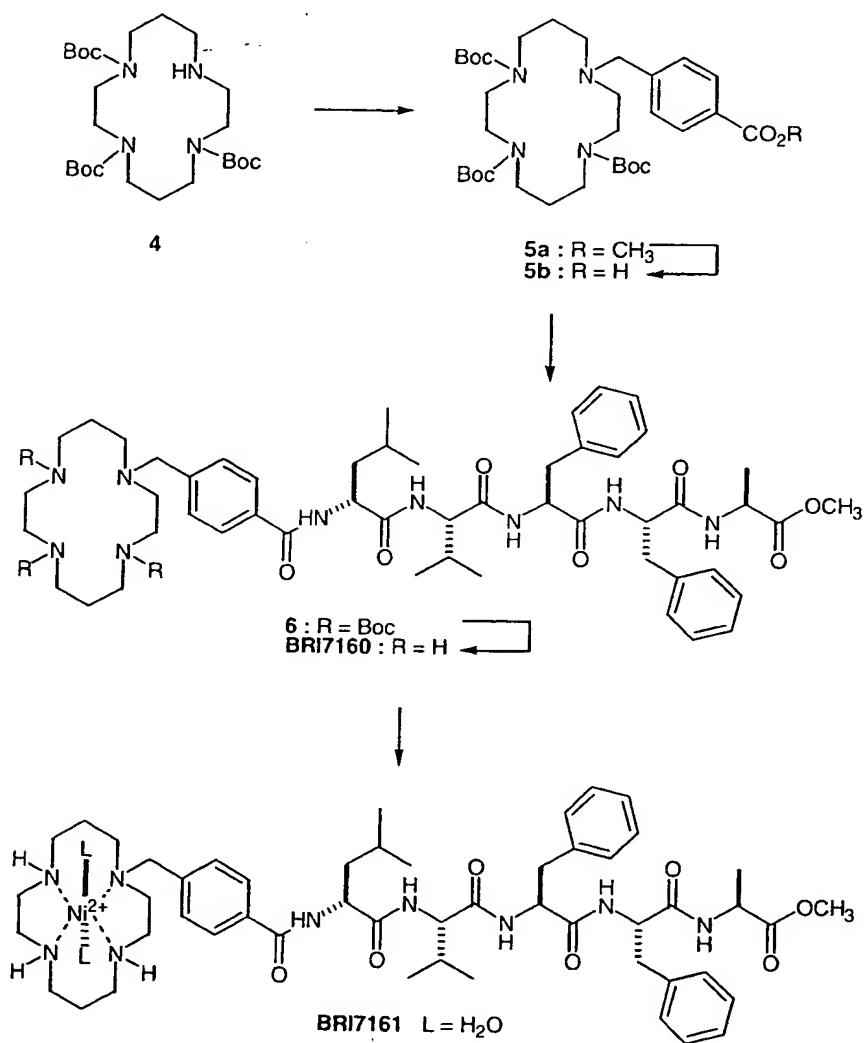
Scheme 4

Compound 4 was prepared following the method of R. Guilard *et al.*, *Bull. Soc. Chim. Fr.*, 1996, 133, 65-73.

A magnetically stirred suspension of compound 4 (430 mg, 0.86 mmol), (4-bromomethyl) methyl benzoate (217 mg, 0.95 mmol), $KHCO_3$ (172 mg) and K_2CO_3 (174 mg) in dry acetonitrile (17 ml) was heated at reflux for 18 h under an atmosphere of nitrogen. The reaction mixture was cooled to room temperature then concentrated under reduced pressure to yield a straw coloured oil which was purified by flash chromatography (silica, 5% methanol/dichloromethane elution). Concentration of the appropriate fractions (R_f 0.38) afforded adduct 5a as a clear, colourless oil.
Mass Spectrum (APCI) *m/z* 649 [$(M+H)^+$, 100%].

A mixture of compound 5a (120 mg, 0.19 mmol), 1M aqueous LiOH (5 ml) and THF (5 ml) was magnetically stirred at room temperature for 16 h. The crude reaction mixture was partitioned between ether and water and the separated aqueous layer was cooled to 0°C prior to acidification to pH 4 with 10% aqueous citric acid solution. Extraction of the aqueous layer with ethyl acetate, followed by drying of the organic fraction with $MgSO_4$ afforded, after concentration under reduced pressure, compound 5b as a viscous, colourless oil.
Mass Spectrum (APCI) *m/z* 633 [(M-H), 100%].

N, N-Diisopropylethylamine (0.17 ml, 0.98 mmol) was added to a magnetically stirred mixture of compound 5b (167 mg, 0.26 mmol), HBTU (132 mg, 0.35 mmol), and BRI7082 (209 mg, 0.29 mmol) in dry DMF (2 ml) under an atmosphere of nitrogen. Stirring was continued at room temperature for 16 h, after which time, the crude reaction mixture was partitioned between ethyl acetate and brine. The separated organic layer was successively washed with 10% aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution and brine before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford compound 6 as a viscous oil.

Mass Spectrum (APCI) m/z 1226 [(M+H)$^+$, 65%].

    A solution of compound 6 (258 mg, 0.21 mmol) in dichloromethane (4 ml) was treated with trifluoroacetic acid (0.1 ml) under an atmosphere of nitrogen and stirring

5 was continued at room temperature for 20 h. The crude reaction mixture was concentrated under reduced pressure to afford a light tan oil which was purified by flash chromatography (silica, 6% methanol/dichloromethane elution). Concentration of the relevant fractions (R_f

10 0.30) afforded BRI7160 as a pale yellow foam.
Mass Spectrum (APCI) m/z 633 [(M-H), 100%].

    A solution of BRI7160 (41 mg, 0.044 mmol) and Ni(OAc)$_2$.4H$_2$O (103 mg, 0.41 mmol) in dry methanol (2 ml) was heated at reflux for 19 h under an atmosphere of

15 nitrogen. The cooled reaction mixture was concentrated under reduced pressure to afford a solid residue. This material was treated with chloroform (4 ml) and the resulting suspension was filtered. Concentration of the filtrate afforded BRI7161 as a foam-like residue.

20 Mass Spectrum (APCI) m/z 1020 [(M+H)$^+$, 30%].

Example 5   **Testing of compound KJB030 for ability to inhibit amyloid β-peptide neurotoxicity _in vitro_**

    Compound KJB030 was dissolved in chelex 100-treated

25 double distilled H$_2$O (CH100-dH$_2$O) at a concentration of 200 μM. Aß1-42 was dissolved in either 200 μM KJB030 solution or in CH100-dH$_2$O. Both solutions contained 200 μM Aß1-42. The Aß1-42 solutions were incubated at ambient temperature (~20-24°C) for 3 days. The solutions were then brought to a

30 physiological state by adding 10x phosphate buffered saline (PBS), pH 7.4, thus bringing samples to 1x PBS. Samples were then incubated at 37°C for 24 hr to induce aggregation of Aß1-42. Samples were subsequently dialysed in microdialysis cups (3500 M_r cut-off) placed in 5 L PBS for

35 24 hr, in order to remove some of the excess KJB030 without loss of Aß1-42.

Dialyzed samples were added to six day old cerebral cortical neuron cultures growing in Neurobasal medium with B27 supplements but without anti-oxidants (Gibco). Samples were diluted into the culture medium to a final

5   concentration of 20 μM Aß1-42. The concentration of the KJB030 was unknown, due to dialysis of the sample; however, the maximum concentration would be 20 μM if no dialysis had occurred. Six wells of each of the following were established: 1; Untreated control cells, 2; Aß1-42 (20 μM),

10   3; KJB030 (dialysed), 4; KJB030+AB1-42 (20 μM, dialysed), 5; vehicle only (PBS). Cultures were treated for 5 days ($37°C/5\%CO_2$,) and culture medium was removed for analysis of cell death.

Determination of cell death was performed using the

15   lactate dehydrogenase (LDH) assay kit (Boehringer Mannheim). This is a measure of the release of LDH, a constitutively expressed, highly abundant enzyme, from dead (lysed) cells. Culture medium was mixed with the reagents from the LDH kit as per the manufacturer's instructions and

20   the level of LDH measured colourimetrically on a spectrophotometric 96 well plate reader at 490 nm. Using the equation supplied with the LDH assay kit, the percentage of LDH release and hence cell death compared to untreated control cultures was determined. The results are

25   shown in Table 1.

Table 1

Results of LDH assay for cell death

Treatment:	% cell death
Untreated control neurons:	0
Vehicle alone (PBS)	-1.18 ± 1.4
KJB030 alone	8.1 ± 2.2
Aß1-42 (20 μM) alone	22 ± 3.5
Aß1-42 (20 μM) + KJB030	3.9 ± 2.5*

*Significantly different from Aß1-42 alone ($p < 0.01$)

Example 6   Demonstration of Reaction of Compound
KJB001 with Aβ1-28

5   1.8 mgs of Aβ1-28 was dissolved in 5 mls DMSO was
mixed with 0.33 mgs of compound KJB001 (~1 equivalent)
dissolved in 0.3 mls DMSO. The mixture was allowed to
stand for 24 hrs before being freeze-dried. The resulting
blue powder was dissolved in 550 μl of an aqueous solution
containing 100 mM NaCl, 50 mM phosphate buffer, pH 6.9. A
10   solution of of 1.8 mgs Aβ1-28 dissolved in 550 μl of the
same solution was used as a control.

       Figure 9(A) shows the 600 MHz ^{1}H NMR spectrum of the
aqueous solution of Aβ1-28 at 271 K. The resonances due to
the C2H protons of His6, 13, 14 are marked with *. Figure
15   9(B) shows the ^{1}H 600 MHz NMR spectrum of the solution of
Aβ1-28 plus KJB001, demonstrating that the peaks due to the
histidine C2H protons had shifted, thus indicating that
KJB001 had reacted with these residues.

20   DISCUSSION

       When copper and iron bind to Aβ, reactive oxygen
species such as peroxide and superoxide are produced. When
copper and zinc bind to Aβ, both induce aggregation, and
copper binding is inhibited by zinc, suggesting that these
25   ions bind to similar binding sites, or share a single
binding site. Zinc, and presumably copper, bind to the
histidine residues of Aβ. Thus a molecule which prevents
the binding of zinc and copper to these histidine residues
has the potential to inhibit Aβ aggregation and to prevent
30   metal-induced neurotoxicity.

       Compounds of the kind described herein have the
potential to bind to histidine residues and therefore to
prevent zinc and copper binding, and so may have
therapeutic value. A model of a cobalt-corrin ring complex
35   bound to Aβ1-40 is shown in Figure 8.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and
5 methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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CLAIMS

1.   A compound which interacts with the β-amyloid peptide in such a way that the N-terminal loop of the peptide (amino acid residues 1-15) is blocked or destabilised, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

2.   A compound according to claim 1 which inhibits binding of Cu^{2+}, Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

3.   A compound according to claim 1 or claim 2 which has a conformation and polarity such that it binds to at least one histidine residue in the N-terminal loop, selected from the group consisting of His6, His13 and His14.

4.   A compound according to claim 3, which binds to at least two histidine residues in the N-terminal loop.

5.   A compound according to claim 4, which binds to at least three histidine residues in the N-terminal loop.

6.   A compound according to any one of claims 1 to 5, which also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

7.   A compound according to any one of claims 1 to 5, which has acidic groups which interact with one or more of the His residues in the N-terminal loop.

8.   A compound according to claim 7, represented as follows:
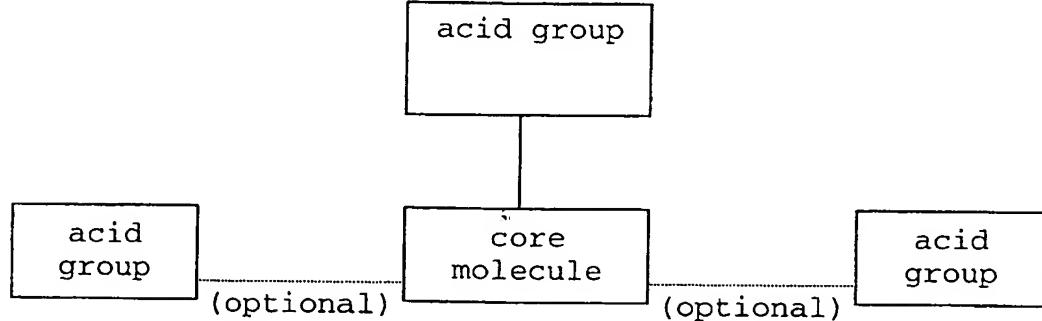
wherein the core molecule has a conformation and polarity such that the acid group(s) interact with one of more of His6, His13 and His14.

9.   A compound according to claim 9, in which the acid group is selected from the group consisting of CO_2H, PO_3H_2, SO_3H, OSO_3H_2, and OPO_3H_2.

10.   A compound according to claim 9, which is a molecule with one to three carboxylic acid groups, the length of the molecule being such that it can be received within the N-terminal loop, and such that at least one carboxyl group is in proximity to at least one of the histidine residues.

11.   A compound according to any one of claims 1 to 10, which is an organic molecule, a peptide or a metal complex.

12.   A compound according to claim 9, which is not a metal complex.

13.   A compound according to claim 9, which has overall hydrophobic character.

14.   A compound according to claim 10, which is able to penetrate the blood-brain barrier.

15.   A compound according to any one of claims 1 to 14, which comprises, or is conjugated to, a targeting moiety.

16.   A compound according to claim 15, in which the targeting moiety is selected from the group consisting of polypeptides, nucleic acids, carbohydrates, lipids, β-amyloid ligands, antibodies, and dyes.

17.   A compound according to claim 15, in which the targeting moiety has a hydrophobic region which interacts with the tail of the β-amyloid peptide.

18.   A compound according to claim 17, in which the targeting moiety comprises a fatty acid molecule.

19.   A compound according to any one of claims 15 to 18, in which the targeting moiety targets the compound to the site defined by residues 15-21 of the β-amyloid peptide.

20.   A compound according to claim 17, in which the targeting moiety is a peptide which comprises a sequence which corresponds to that of residues 15-21 of the β-amyloid peptide.

21. A compound according to any one of claims 15 to 20, in which the inhibitor-targeting moiety complex is able to penetrate the blood-brain barrier.

22. A method of selecting or designing a compound which inhibits the binding of metal ions to the N-terminal loop of the β-amyloid peptide, which method comprises the steps of

(i) selecting or designing a compound which has a conformation and polarity such that it binds to at least one, more preferably at least two and more preferably three amino acids in the N-terminal loop selected from the group consisting of His6, His 13 and His14; and

(ii) testing the compound for the ability to inhibit binding of metal ions to the N-terminal loop of the β-amyloid peptide.

23. A method according to claim 22, in which the compound binds to at least two histidine residues in the N-terminal loop.

24. A method according to claim 23, in which the compound binds to at least three histidine residues in the N-terminal loop.

25. A method according to any one of claims 22 to 24, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11. Preferably the compound inhibits binding of both Cu^{2+} and Zn^{2+} ions, but not Mg^{2+} or Ca^{2+} ions.

26. A method according to claim 26, in which the compound inhibits binding of Cu^{2+}, Zn^{2+} and Fe^{3+} ions, but not Mg^{2+} or Ca^{2+} ions.

27. A method according to any one of claims 22 to 26, in which the compound has overall hydrophobic character.

28. A method according to claim 27, in which the compound is able to penetrate the blood-brain barrier.

29. A compound which inhibits the binding of metal ions to the N-terminal loop of the β-amyloid peptide, wherein

the compound is obtained by a method according to any one of claims 22 to 28.

30.   A composition comprising a compound according to any one of claims 1 to 21 or claim 29, together with a

5   pharmaceutically acceptable carrier.

31.   A method of inhibiting the binding of one or more metal ions to the β-amyloid peptide, or of inhibiting the aggregation of β-amyloid peptide, which method comprises the step of exposing the peptide to a compound which blocks

10   or destabilises the N-terminal loop of the peptide, thereby inhibiting the binding of one or more metal ions to at least one histidine residue within the N-terminal loop.

32.   A method according to claim 31, in which the compound has a conformation and polarity such that it binds to at

15   least one histidine residue in the N-terminal loop of the β-amyloid peptide, selected from the group consisting of His6, His13 and His14.

33.   A method according to claim 32, in which the compound binds to at least two histidine residues in the

20   N-terminal loop.

34.   A method according to claim 33, in which the compound binds to at least three histidine residues in the N-terminal loop.

35.   A method according to any one of claims 31 to

25   34, in which the compound also binds to at least one additional amino acid in the N-terminal loop, selected from the group consisting of Asp7, Tyr10, and Glu11.

36.   A method according to any one of claims 31 to 35, in which the compound inhibits binding of Cu^{2+}, Zn^{2+} and Fe^{3+}

30   ions, but not Mg^{2+} or Ca^{2+} ions.

37.   A method according to any one of claims 31 to 36, in which the compound is a complex of Mn, Fe, Co, Ni, Cu, Zn, Ru, Pd, Ag, Cd, Pt, Au, Rh or Hg, with the proviso that the compound is not haemin or haematin.

35   38.   A method according to any one of claims 31 to 37, in which the compound comprises, or is conjugated to, a targeting moiety.

39.   A method according to claim 38, in which the targeting moiety targets the compound to the site defined by residues 15-21 on the β-amyloid peptide.

40.   A method according to any one of claims 31 to 39, in which the inhibition of binding of one or more metal ions to the β-amyloid peptide occurs *in vivo*.

41.   A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises the step of administering a compound according to any one of claims 1 to 21 or a pharmaceutical composition according to claim 30 to a subject in need of such treatment.

42.   A method of prevention, treatment or alleviation of Alzheimer's disease, which method comprises inhibiting the binding of one or more metal ions to the β-amyloid peptide, or inhibiting the aggregation of β-amyloid peptide, by the method of claim 40.